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CJC

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner for Patents, P.O. Box 1450 Alexandria, VA 22313 on April 18, 2006.

Frank C Eisenschenk

Frank C. Eisenschenk, Ph.D., Patent Attorney

REQUEST FOR CERTIFICATE OF CORRECTION UNDER 37 CFR 1.322
Docket No. G-069US01REG
Patent No. 7,015,030

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants	:	Yves Fouillet, Claude Vauchier, Jean-Frederic Clerc, Christine Peponnet
Issued	:	March 21, 2006
Patent No.	:	7,015,030
For	:	Microfluidic Devices and Uses Thereof in Biochemical Processes

Mail Stop Certificate of Corrections Branch
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

REQUEST FOR CERTIFICATE OF CORRECTION UNDER 37 CFR 1.322 (OFFICE MISTAKE)

Sir:

A Certificate of Correction (in duplicate) for the above-identified patent has been prepared and is attached hereto.

In the left-hand column below is the column and line number where errors occurred in the patent. In the right-hand column is the page and line number in the application where the correct information appears.

Patent Reads:	**Application Reads:**
Column 23, line 38:	Page 35, lines 22 and 23:
"Clin Microbiol"	--J Clin Microbiol--

Column 23, lines 41 and 42:	Page 35, line 25:
"Clin Microbiol"	--J Clin Microbiol--
Column 23, line 45:	Page 35, line 27:
"Clin Microbiol"	--J Clin Microbiol--
Column 42, line 63:	Page 64, line 12:
"enzymes-occurs."	--enzymes occurs.--
Column 54, line 10:	Page 81, line 5:
"that 35"	--that ~35--.

A true and correct copy of pages 35, 64, and 81 of the specification as filed which support Applicants' assertion of the errors on the part of the Patent Office accompanies this Certificate of Correction.

Approval of the Certificate of Correction is respectfully requested.

Respectfully submitted,

Frank C Eisenschenk

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FCE/lbm

Attachments: Copy of pages 35, 64, and 81 of the specification

APR 27 2006

An example a protocol for amplification by PCR which has been carried in a microfluidic substrate according to the invention is provided in Example 1.

Temperature cycling and genetic analysis

In genetic analysis, numerous protocols exist which require temperature cycling. Amplification techniques which are derived from the PCR are known, such as RT-PCR, allele-specific PCR and Taq Man PCR (White, B.A., Methods Mol Biol 67:481-486 (1997); Delidow, B.C. *et al.*, Methods Mol Biol 58: 275-292 (1996), the contents of which are incorporated herein by reference in their entireties). Ligase chain reaction (LCR) techniques are also well known, including LCR, gap LCR, asymmetric gap LCR, reverse transcription LCR (RT-LCR), the oligonucleotide ligation assay (OLA) and PCR-OLA (Nikiforov, T., Anal Biochem 225: 201-209 (1995); Marshall, R.L., PCR Methd Appl 4: 80-84 (1994); Nickerson, D.A. *et al.*, Proc Natl Acad Sci USA 87: 8923-8927 (1990), the contents of which are incorporated herein by reference in their entireties). Cyclic sequencing reactions using clones or PCR reactions are known. Cyclic microsequencing (single nucleotide primer extension) reactions are known (Cohen, D., International patent publication no. WO 91/02087, incorporated herein by reference in its entirety). Further exemplary protocols include reverse-transcriptase and nested PCR (RT-nested PCR) of expressed sequences, followed by cycle sequencing (Happ *et al.*, Vet Immunol Immunopathol, 69: 93-100, (1999), incorporated herein by reference in its entirety); a degenerate PCR technique can be used as a first step to identify and amplify the actual sequence when it is not completely known (Harwood *et al.*, J Clin Microbiol 37: 3545-3555, (1999), incorporated herein by reference in its entirety); random amplified DNA (RAPD) analysis, often useful as a exploratory approach (Speijer et al, J Clin Microbiol 37: 3654-3661, (1999), incorporated herein by reference in its entirety); and arbitrarily primed PCR (AP-PCR), often useful as a preliminary step to find potential polymorphisms (Jonas *et al.*, J Clin Microbiol 38: 2284-2291, (2000), incorporated herein by reference in its entirety).

Although various devices have been designed for implementing chemical, biochemical, and biological protocols comprising steps that include at least one thermal cycle, these devices have serious drawbacks. Advantages provided by the present invention are apparent from comparing other devices with the present invention.

glycoprotein is introduced to the channel thorough a reagent basin or reservoir. The first glycoprotein sample moves to the first temperature regulated zone (37°C) and flows through said temperature regulated zone for a given time, during which the oligosaccharides are realeased from the glycoprotein. While still in said first temperature regulated zone, the temperature of this temperature regulated zone is changed with a rapid transition from 37°C (an optimal temperature for enzyme activity) to, *e.g.*, 94°C (for 10 minutes) to inactivate the enzyme. The first glycoprotein sample exits the first temperature regulated zone, the temperature of this first temperature regulated zone is rapidly changed to 37°C and the second glycoprotein sample enters the first temperature regulated zone. Thus the first temperature regulated zone is cycling between 37°C, where enzymatic cleavage of polysaccharides or proteins occurs, and 94°C, where inactivation of enzymes occurs. After the oligosaccharides are released from a glycoprotein, 1μl of a mixture of a fluorescent dye (*e.g.*, amino-pyrene three sulfonic acid (APTS) 20mM) and $NaCNBH_3$ (0.4M) are added to the sample. The mixture enters a second temperature regulated zone that is equilibrated at 94°C. The sample moves through the second temperature regulated zone during a time period of 1 hour. The oligosaccharides from a glycoprotein are labeled by the fluorescence dye in the second temperature regulated zone. Finally, the sample is either collected and analyzed off-line by capillary electrophoresis with laser induced fluorescence detection and MALDI/TOF mass spectrometry, or it is injected into an electrophoretic microfluidic device that is integrated with the microfludic device used for the temperature treatment, and analyzed on-line.

Tryptic digest

In another example, proteins from cells are separated by 2D-gel electrophoresis. A given glycoprotein is transferred from a gel into a solution and introduced into a channel of the microfluidic substrate. Since this substrate has channels in parallel many different proteins can be injected in parellel, and in series. An enzyme (*e.g.*, pepsin, trypsin) is introduced to the channel via a first reagent feed basin or reservoir. The first sample enters into a first temperature regulated zone (37°C) and it flows through this first temperature regulated for a given time, during which the proteins are cleaved and peptide molecules are formed. While still in the first temperature regulated zone, the temperature of this temperature regulated zone

the molecular beacon probes have similar Tms in order to keep the same cycling temperatures for all the different reactions. The different reaction mix are injected one after the other in the device, however when injected the mix is injected in all the channels in parallel.

The flow rates in the substrate is 8.2µl/hour, such that ~35 cycles are carried out on 2cm of the PCR PCR temperature regulated cycling zone.

This reaction mixture can be treated before injection into a device at 94°C for 10 minutes if necessary to activate the polymerase. It is run through a zone which is cycled, in which a cycle consists of a step of 20 sec. at 94°C, then 20 sec. at 55°C and 20 sec. at 72°C, for a duration corresponding to 35 cycles.

Once the PCR reaction flows out of the PCR temperature regulated zone, the PCR is completed, the reaction can then pass a thermostated detection zone consisting of a fluorimeter adapted to the molecular beacon wave length and continuous flow detection. In an other embodiment the PCR reactions can be collected from the oulet basin and analyzed on an ABI Prism 7700 Sequence Detection System according to the manufacturers instructions.

Although this invention has been described in terms of certain preferred embodiments, other embodiments which will be apparent to those of ordinary skill in the art in view of the disclosure herein are also within the scope of this invention. Accordingly, the scope of the invention is intended to be defined only by reference to the appended claims. All documents cited herein are incorporated herein by reference in their entirety.

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 7,015,030

APPLICATION NO.: 09/627,647

DATED : March 21, 2006

INVENTORS : Yves Fouillet, Claude Vauchier, Jean-Frederic Clerc, Christine Peponnet

It is certified that errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 23,
Line 38, "Clin Microbiol" should read --J Clin Microbiol--.
Lines 41 and 45, "Clin Microbiol" should read --J Clin Microbiol--.

Column 42,
Line 63, "enzymes-occurs." should read --enzymes occurs.--.

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Line 10, "that 35" should read -- that ~35--.

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